PATENT
NY-HUBR 1230-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)
Chris RUNDFELD et al.	) Group Art Unit: 1617
Application No.: 10/680,459	) Examiner: D. R. CLAYTOR
Filed: October 6, 2003	) Confirmation No.: 4494
For: USE OF DIHYDROIMIDAZOLONES FOR THE TREATMENT OF DOGS	)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF DR. HOLGER VOLK UNDER 37 C.F.R. § 1.132

I, Dr. Holger Volk, DVM, PhD, DipECVN, MRCVS, do hereby make the following declaration:

1. I am employed by the University of London, the Royal Veterinary College. I am Lecturer in Veterinary Neurology & Neurosurgery and head of the aforementioned service at the Royal Veterinary College. I am also active as team neurologist in the first epilepsy clinic for animals in Europe at the Queen Mother Hospital for Animals.

2. I have read and am familiar with the subject patent application. I am also familiar with the references cited by the Examiner during the prosecution of the patent application including Bialer et al., Epilepsy Research 43 (2001), 11-58, which describes the activity of AWD

131-138 in animal seizure models, and the other cited references. I am also familiar with the body of literature dealing with epilepsy treatment in animals.

3. I have read and am familiar with the arguments previously advanced by both the applicants and the Examiner concerning the use of AWD 131-138 for the treatment of idiopathic epilepsy in dogs.

4. The available treatment options for canine idiopathic epilepsy are very limited, even today. Phenobarbital,primidone and potassium bromide are established for treatment of canine epilepsy, although the use of primidone is not used as frequent anymore due to its increased risk of hepatotoxicity. Phenobarbital and primidone are not marketed in most countries for the treatment of canine epilepsy. Potassium bromide is also not licensed as a drug in many countries but as a food supplement. Rather, human medications containing phenobarbital or primidone are administered to dogs with idiopathic epilepsy.

5. Other human antiepileptic medications were already available at the time of the invention, but these medications are not suitable for the treatment of dogs with idiopathic epilepsy. Indeed, most of these drugs have been tested on dogs and I have been involved in some of this work. Astonishingly, despite the fact that these medications, including carbamazepine, valproic acid, phenytoine, and vigabatrin, for example, are very effective anti-epileptic drugs for humans, they were not effective in treating dogs. In most cases, they did not provide effective against seizures. In the case of vigabatrin, the administration to dogs resulted in unexpected toxic effects presenting as neuropathology (Yarrington et al., Sequential

neuropathology of dogs treated with vigabatrin, a GABA-transaminase inhibitor., Toxicol Pathol. 1993; 21:480-9, see **Exhibit 1**). Vigabatrin therefore can not be used in dogs since it is toxic.

6. The reason for failure of these other drugs in my opinion is related to the unique physiology of canines. Dogs have a high metabolizing capacity, which leads to short half lives of many drugs. This metabolizing capacity is even further strengthened upon repeated administration of a medication since induction of the metabolizing enzymes results in an even lower achievable plasma level and an even shorter half life. An example of these characteristics, i.e. the short half life and the enzyme induction, is described in Schicht et al, Pharmacokinetics of oxcarbazepine in the dog, J Vet Pharmacol Ther. 1996;19:27-31 (**Exhibit 2**). In this paper, oxcarbazepine, a derivative of carbamazepine, used for treatment of human epilepsy, was tested in dogs. While the initial half life was in the range of 4 hours (which is not sufficient to allow for a full day exposure after once or twice daily treatment), this half life declined within 3 days, upon repeated administration (three times a day) to 1-2 hours rendering the drug useless for treatment of dogs. The authors conclude that oxcarbazepine, compared with former results with carbamazepine, offers no advantage for the treatment of epileptic dogs (carbamazepine was tested previously by the authors and was shown to also have a short half life and strong enzyme induction in dogs).

7. Even in the case of phenobarital, which is considered effective for the treatment of canine epilepsy, it is necessary to increase the dosage dramatically throughout the course of the treatment, to ensure adequate plasma levels. Normally, one would counteract such a short half life and low plasma levels with increased doses, administered as modified release tablets, which release the active ingredient over many hours during enteral passage. Indeed, many

human antiepileptics including carbamazepine and valproic acid are used as modified release tablets. Enteral passage in the dog is very rapid, making it impossible to use modified release tablets to ensure a full day drug exposure. Both the rapid metabolism of many drugs compared to humans and rapid enteral passage preventing the use of retarded formulations developed for humans prevent the necessary build up of active plasma concentrations of the drug over the course of a full day.

8. For these reasons, the fact that a compound is active in humans is not predictive for treatment of canine idiopathic epilepsy. This is true for drugs which are already available for human idiopathic epilepsy, as well as for any new chemical entity which has only been tested in individual seizure models, which represent models of induced seizures. In such models, the compound is administered at a given point in time frame relative to the induction of the seizure. The seizure is then induced by an external stimulus, such as sound, electroshock or administration of chemoconvulsants at the predetermined optimal time to show the best possible effect. Such models may indicate that symptomatic epileptic seizures may be prevented if the drug is administered as a single dose, at a predetermined time before the inducing event, but such models do not suggest efficacy in canine idiopathic epilepsy. Dogs suffering from canine idiopathic epilepsy may experience seizures at any time throughout the day. There are no reliable predictors when a seizure can be expected. Therefore, a treatment can only be effective if after drug administration anticonvulsant activity can be ensured for several hours after administration. The duration of action must be in line with a dosing regime which is in a best case once daily or, alternatively twice daily or three times daily which represents the highest acceptable frequency for repeated dosing. Due to the high metabolic capacity of the canine

which often is further induced by drugs effective plasma levels are often not reached for a sufficiently long period of time. This can not be compensated by the use of modified release formulations since the rapid enteral passage in dogs prevents the adequate slow long lasting absorption of drugs released from these formulations during several hours.

9. In conclusion, as an experienced clinician in the field of canine epilepsy, I would not have selected AWD 131-138 as an effective drug for the treatment of canine idiopathic epilepsy based on the data in the references cited in the patent application and especially based on the data published by Bialer et al.. In my opinion, it is unexpected that AWD 131-138 is effective in the treatment of canine idiopathic epilepsy.

10. I hereby declare that all statements made herein where done on the basis of my best knowledge and that all statements made are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 18/08/03

By: Dr. Holger VOLK

Curriculum Vitae

I studied veterinary medicine at the Veterinary School of Hanover, Germany and at the Ecole Nationale Vétérinaire de Lyon, France, which was partly funded by the German National Academic Foundation. I graduated from the Veterinary School of Hanover in June 2001.

Following this, I started a German National Academic Foundation funded 3-year PhD programme in veterinary science at the Department of Pharmacology, Toxicology, and Pharmacy, Veterinary School of Hanover.

In July 2004, I finished my study about the underlying mechanisms of pharmacoresistant epilepsy. I was awarded with a PhD, which was rewarded with the Domagk Prize of the pharmaceutical company Bayer. I then completed an internship and a residency in Neurology and Neurosurgery at The Royal Veterinary College in September 2007. In September 2008, I successfully passed the examination for the Diploma of the European College of Veterinary Neurology (ECVN) and obtained the title DipECVN (Recognized European Specialist in Veterinary Neurology).

I am head of the Neurology & Neurosurgery service and currently a Lecturer in Neurology and Neurosurgery at the Royal Veterinary College. My main research interests are Chiari-like malformation and syringomyelia in Cavalier King Charles Spaniel and canine and feline epilepsy, especially the underlying mechanisms of the disorder itself and of pharmacoresistant epilepsy.

Selected Publications

VOLK, H. A., MATIASEK, L. A., LUJAN FELIU-PASCUAL, A., PLATT, S. R. & CHANDLER, K. E. (2008) The efficacy and tolerability of levetiracetam in pharmacoresistant epileptic dogs. Vet J 176, 310-319. PubMed ID 17468024

BRANDT, C., GLIEN, M., GASTENS, A. M., FEDROWITZ, M., BETHMANN, K., VOLK, H. A., POTSCHKA, H. & LÖSCHER, W. (2007) Prophylactic treatment with levetiracetam after status epilepticus: lack of effect on epileptogenesis, neuronal damage, and behavioral alterations in rats. Neuropharmacology 53, 207-221. PubMed ID 17585956

WESSMANN, A., VOLK, H. A., SHELTON, G. D., CHANDLER, K. E., BAINES, S. & CAPPELLO, R. (2006) Portosystemic shunt associated with severe episodic weakness. J Vet Intern Med 20, 1042-1044. PubMed ID 16955838

VOLK, H. A., ARABADZISZ, D., FRITSCHY, J. M., BRANDT, C., BETHMANN, K. & LÖSCHER, W. (2006) Antiepileptic drug-resistant rats differ from drug-responsive rats in hippocampal neurodegeneration and GABA(A) receptor ligand binding in a model of temporal lobe epilepsy. Neurobiol Dis 21, 633-646. PubMed ID 16256358

HOFFMANN, K., GASTENS, A. M., VOLK, H. A. & LÖSCHER, W. (2006) Expression of the multidrug transporter MRP2 in the blood-brain barrier after pilocarpine-induced seizures in rats. Epilepsy Res 69, 1-14. PubMed ID 16504477

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VOLK, H. A., BURKHARDT, K., POTSCHKA, H., CHEN, J., BECKER, A. & LÖSCHER, W. (2004) Neuronal expression of the drug efflux transporter P-glycoprotein in the rat hippocampus after limbic seizures. Neuroscience 123, 751-759. PubMed ID 14706787

POTSCHKA, H., VOLK, H. A. & LÖSCHER, W. (2004) Pharmacoresistance and expression of multidrug transporter P-glycoprotein in kindled rats. Neuroreport 15, 1657-1661. PubMed ID 15232302

BRANDT, C., VOLK, H. A. & LÖSCHER, W. (2004) Striking differences in individual anticonvulsant response to phenobarbital in rats with spontaneous seizures after status epilepticus. Epilepsia 45, 1488-1497. PubMed ID 15571506

BRANDT, C., GLIEN, M., POTSCHKA, H., VOLK, H. & LÖSCHER, W. (2003) Epileptogenesis and neuropathology after different types of status epilepticus induced by prolonged electrical stimulation of the basolateral amygdala in rats. Epilepsy Res 55, 83-103. PubMed ID 12948619

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Sequential Neuropathology of Dogs Treated with Vigabatrin, a GABA-Transaminase Inhibitor*

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ABSTRACT

Vigabatrin (Sabril®) is a γ-aminobutyric acid-transaminase (GABA-T) inhibitor that is effective in the treatment of certain types of drug-resistant or uncontrolled epilepsy but is known to cause microscopic vacuolation (intramyelinic edema) in the brains of treated rats, mice, and dogs. The effects of high oral doses (300 mg/kg/day) of vigabatrin administered orally to Beagle dogs were studied during treatment weeks 1–12 and recovery weeks 13, 14, 16, 20, 24, and 28. Emesis, loose stools, and anorexia and 3 drug-related deaths were observed during the first 4 wk of treatment but were virtually nonexistent thereafter because of adaptation to the drug aided by food supplementation. In more sensitive areas of the brain (columns of the fornix, thalamus, and hypothalamus), microscopic quantitative differences between background vacuolation in controls and drug-related vacuolation in treated dogs could be delineated after 4 wk, generally reached highest levels of severity between 8 and 12 wk, and were reversible upon cessation of dosing. Inhibition of brain GABA-T and elevation of brain GABA were noted after 1 wk of treatment. During the course of treatment vigabatrin ranged between 4–17 nmol/ml (plasma) and 42–1,570 nmol/ml [cerebrospinal fluid (CSF)] while CSF GABA concentrations were 4–32 nmol/ml (treated dogs) and 0.1–0.6 nmol/ml (control dogs). Although the cause of vigabatrin-induced microvacuolation is unknown, the results of the study demonstrated that GABA-T inhibition with subsequent GABA elevation occurred within the first week of treatment and was followed by the onset of detectable microvacuolation several weeks later.

Keywords. γ-vinyl GABA; brain vacuolation; intramyelinic edema; reversibility

INTRODUCTION

Vigabatrin [4-amino-5-hexenoic acid, γ-vinyl γ-aminobutyric acid (GABA), Sabril®] is an enzyme-activated irreversible inhibitor of GABA transaminase (GABA-T) (18, 19), the enzyme responsible for the degradation of GABA, a major inhibitory neurotransmitter. Because vigabatrin-induced elevation of brain GABA has been shown to prevent seizures in experimental animal models (24–26, 31), testing has been extended to human patients with difficult to control epilepsy. Currently, vigabatrin has demonstrated efficacy in patients with uncontrolled or drug-resistant focal epilepsy with complex partial seizures (3, 12, 29).

Toxicity studies with vigabatrin have demonstrated microscopic brain vacuolation, most notably in white matter tracts, as the main toxic effect in mice, rats, and dogs given dosages of 30–300 mg/kg/day in subacute and chronic tests (1, 4, 5, 9, 10, 32, 37). Cynomolgus monkeys treated with 300 mg/kg/day for 16 mo exhibited minimal microvacuolation, which was considered equivocal since it was not noticeably different from background observed in controls (4, 9, 10). Other monkeys receiving 50 or 100 mg/kg/day for up to 6 yr did not exhibit any adverse effects (9). Ultrastructural examination of the brains from treated rats (5, 9) and dogs (1) demonstrated that the microvacuoles occurred as a result of splitting of the outer lamellar layers of the myelin sheaths at the intraperiod line, findings compatible with intramyelinic edema.

The presence of intramyelinic edema during preclinical animal toxicity studies of vigabatrin has created safety concerns for human patients. Because of these concerns considerable effort has been made to develop noninvasive methods that can detect the presence of this intramyelinic edema in dogs. Recent, biweekly electrophysiological testing of dogs has shown that 300 mg/kg/day of vigabatrin given for 15 wk caused significant changes in conduction of myelinated fiber tracts of the central nervous system characterized by slowing of somatosensory and

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visual evoked potentials (32). Earliest changes in these evoked potentials appear to occur between 4 and 6 wk; however, statistically significant differences were not evident until 8–10 wk. In these same chronically administered dogs, magnetic resonance imaging (MRI) of brains taken at the end of the treatment period detected changes both *in vivo* and *ex vivo* in areas (thalamus, hypothalamus, and columns of the fornix) where intramyelinic edema was prominent histologically (37).

The purpose of the present investigation was to determine microscopically the time-course for the onset and reversibility of brain vacuolation in vigabatrin-treated dogs with particular emphasis on structures demonstrating marked sensitivity. This was done so that the time sequence of these changes can be correlated with evoked potential and MRI changes. Related effects including clinical signs, brain biochemistry, and pharmacokinetic data were also evaluated. The results of *ex vivo* MRI scans of these same brains will be reported elsewhere (28).

Materials and Methods

Experimental Animals. Fifty-four male and 54 female Beagles (Hazelton Research Products, Inc., Kalamazoo, MI) identified by chest tattoo, collar tag, and cage card were individually housed in fenced runs in light- and temperature-controlled rooms and conditioned to their laboratory environment for a minimum of 37 days. Each animal was fed *ad libitum* dry dog food (Certified Canine Diet® No. 5007, Purina Mills, Richmond, IN) or if they became anorexic while on treatment, canned dog food (Ken-L-Ration®, The Quaker Oats Company, Chicago, IL). Drinking water (deionized) was provided continuously by means of automatic waters. All animal care and procedures were in conformity with the NIH *Guide for the Care and Use of Laboratory Animals* (7).

Experimental Design. Eighteen experimental groups were created by assigning 2 control dogs (1 male, 1 female) and 4 dogs treated with 300 mg/kg/day (2 males, 2 females) to each group. The dose of 300 mg/kg, which was given daily in gelatin capsules, was chosen because it has previously been shown to cause marked vacuolation in the brains of dogs after 3 months of treatment (1, 9, 32). To achieve representative groups, animal assignments were based on history, sex, weight, and pretest clinicopathological data. A group of dogs was necropsied weekly during treatment period weeks 1–12 and after recovery weeks 13, 14, 16, 20, 24, and 28.

Clinical Observations. The dogs were observed at least twice daily for any physical abnormality and clinical signs. Physical examinations were also given pretest and prior to scheduled necropsies.

Clinical Pathology. Blood samples from fasted dogs were collected by venipuncture for hematology and clinical chemistry determinations during pretest and before sacrifice. Hematological parameters consisted of erythrocyte counts, leukocyte counts, platelet counts, hematocrit, and hemoglobin (determined by an Ortho ELT-8 Hematology Analyzer®, Ortho Diagnostic Systems, Westwood, MA); morphological and differential evaluation of erythrocytes (nucleated cells, reticulocytes, Heinz bodies) performed manually using blood smears; and prothrombin and partial thromboplastin times (measured by an Electra 600 Automatic Coagulation Timer®, Medical Laboratory Automation, Inc., Pleasantville, NY). Serum chemistry measurements (Dacos Analyzer®, Coulter Electronics, Inc., Hialeah, FL) included: chloride, carbon dioxide, total and direct bilirubin, total protein, albumin, calcium, inorganic phosphorus, glucose, blood urea nitrogen, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, potassium, cholesterol, triglycerides, and creatinine.

Pathology. With the exception of 2 dogs that died on study, all dogs were sacrificed under anesthesia (Surital®, Parke-Davis, Division of Warner-Lambert Co., Morris Plains, NJ) and then exsanguinated prior to necropsy. Immediately, the dorsal calvarium was removed exposing the brain. After a small specimen of brain tissue (left temporal lobe) was removed for biochemical analysis, the remaining intact brain was promptly placed in 20% buffered formalin. The cervical and lumbar spinal cord segments, dorsal root ganglion, stellate ganglion, caudal cervical ganglion, celiac ganglion, and peripheral nerve (sciatic nerve) were then dissected and placed in 20% formalin while both eyes were placed in Davidson's fixative. Following fixation, tissue specimens were processed, embedded in paraffin, sectioned at 5–6 mm, mounted on glass slides, and stained with hematoxylin and eosin for microscopic examination. To insure a comprehensive evaluation of the brain, coronal cross sections were made of the following levels: (A) optic chiasma; (B) hypothalamus, columns of the fornix, and optic tract; (C) lateral geniculate body and hippocampus; and (D) cerebellum containing roof nuclei. Independent microscopic evaluation by 2 pathologists was conducted in a blinded fashion since tissues were coded by a designated histology technician so that the identities of the dogs were unknown as to actual dog study number, dose, and treatment/recovery week. Since the expected neuropathology of vigabatrin-treated dogs is primarily vacuolation, special detail was given to defining and grading this lesion. Vacuoles lined by endothelial cells (dilated capillaries) were eliminated from consideration; only sharply

outlined vacuoles were evaluated. Severity grade of vacuolation based on percentage brain structure involvement (0, <1%; 1, 1–20%; 2, 21–40%; 3, 41–60%; 4, 61–80%; and 5, >81%) as well as distribution (focal, multifocal, or diffuse) were recorded. After a consensus determination of all vacuolation findings by the two pathologists, the results were decoded and properly identified. Study findings were then retabulated as to actual dog and treatment/recovery week. All other analyses (see below) were also done in a blinded fashion.

Concentrations of Plasma Vigabatrin and Cerebrospinal Fluid Vigabatrin and GABA. Prior to a scheduled necropsy (24 hr postdosing), blood (5–7 ml) was collected, transferred to a glass tube containing EDTA (Vacutainers®, Becton-Dickinson and Co., Rutherford, NJ) and placed in ice until centrifuged a short time later. Following centrifugation, plasma was pipetted into small screw-cap scintillation tubes, frozen, and temporarily stored at −70°C. Plasma vigabatrin levels were determined using a high-performance liquid chromatography (HPLC) method with fluorometric detection (33) for the purpose of establishing the degree of absorption of the drug.

Cerebrospinal fluid (CSF) was aspirated (21-gauge needle/3-cc syringe) 24 hr postdosing from the cisterna magna of the anesthetized dogs just prior to necropsy. CSF vigabatrin and GABA concentrations were determined by gas chromatographic (GC)/positive chemical ionization mass spectrometry (MS) as previously described (9). GABA conjugates were acid hydrolyzed prior to GC/MS analysis so that total GABA (free and conjugated) could be determined. CSF GABA has been shown to correlate well with changes in brain GABA concentrations (2, 13) while CSF vigabatrin indicates drug passage through the blood–brain barrier.

Analysis of Brain Tissue for GABA, GABA-T, and GAD. A superficial section (less than 0.2 g) of the left temporal lobe of the cerebrum was removed within 3–4 min after exsanguination of anesthetized dogs and quickly frozen in liquid nitrogen. These samples were stored frozen at −80°C until assayed for GABA, GABA-T, and GAD (glutamate decarboxylase) according to methods previously used for brains of mice, rats, and monkeys (18–21). GAD is the enzyme responsible for the synthesis of GABA in GABAergic neurons (12, 20).

Statistical Analysis. Quantitative differences between treatment-related vacuolation and background found in controls were evaluated by analysis of variance methods (34) at each time point. Time-related trends in vacuolation severity were also evaluated by analysis of covariance (34), which included effects of sex and week of sacrifice.

TABLE I.—Sensitivity of evaluated brain structures to vacuolation.

Brain structure	Highest average grade of severity (0–5)		Occurrence (week)	
	Treated	Control	Treated	Control
Columns of the fornix	5.0	1.0	11	8
Medial forebrain bundle	4.5	0.5	11	8
Stria medullaris	3.8	0.5	12	8
Fimbria of the fornix	3.5	0.5	7	8
Anterior commissure	3.5	0.5	5	10
Thalamus	3.3	0.0	12	–
Hypothalamus	3.3	1.0	8	5
Dorsal fornix	2.8	1.0	12	11
Hippocampus	2.5	0.5	12	1
Optic chiasma	2.5	0.5	12	5
Cerebellum	2.3	2.0	9	5
Medial geniculate body	2.0	0.0	12	–
Habenular nucleus	2.0	0.5	11	4
Pretectal nucleus	2.0	0.0	10	–
Posterior commissure	2.0	0.0	8	–
Optic tract	1.5	1.0	8	12
Corpus callosum	1.3	1.0	12	5
Lateral geniculate body	1.0	0.0	9	–
Mammillothalamic tract	1.0	0.0	6	–
Cerebral peduncle	0.8	0.5	12	3
Lumbar spinal cord	0.8	1.0	10	8
Eyes	0.8	1.0	4	4
Cortex	0.5	0.0	7	–
Medial lemniscus pathway	0.3	0.0	12	–
Dorsal root ganglion	0.3	0.0	12	–
Celiac ganglion	0.3	0.0	5	–
Internal capsule	0.0	–	–	–
Caudate nucleus	0.0	0.0	–	–
Cervical spinal cord	0.0	0.5	–	9
Sciatic nerve	0.0	0.0	–	–
Stellate ganglion	0.0	0.0	–	–
Caudal cervical ganglion	0.0	1.0	–	2

RESULTS

Clinical Observations

Within the first week of treatment most treated dogs became anorexic. Between the second and fourth week of dosing, 2 treated dogs died and 1 was sacrificed because of its moribund condition. During this period of treatment, clinical pathological evaluation of many of the sacrificed dogs demonstrated increased cholesterol and triglycerides. These findings are likely related to anorexia, which appeared to be a major contributory factor in the cause of death or morbidity. To encourage eating, anorexic dogs were first fed canned dog food. However, a liquid slurry of canned dog food was given by gavage to any dog not forming stools, not eating within a 24-hr period, and/or losing body weight (≥10%). After forced feeding began, no further deaths occurred. Additional sporadic observations included emesis, soft stools, and diarrhea, which may have been treatment related. After about 1 mo of dosing, the clinical observations became less frequent and severe. By the end of treatment and during 1–16 wk of recovery, no signs of toxicity were noted.

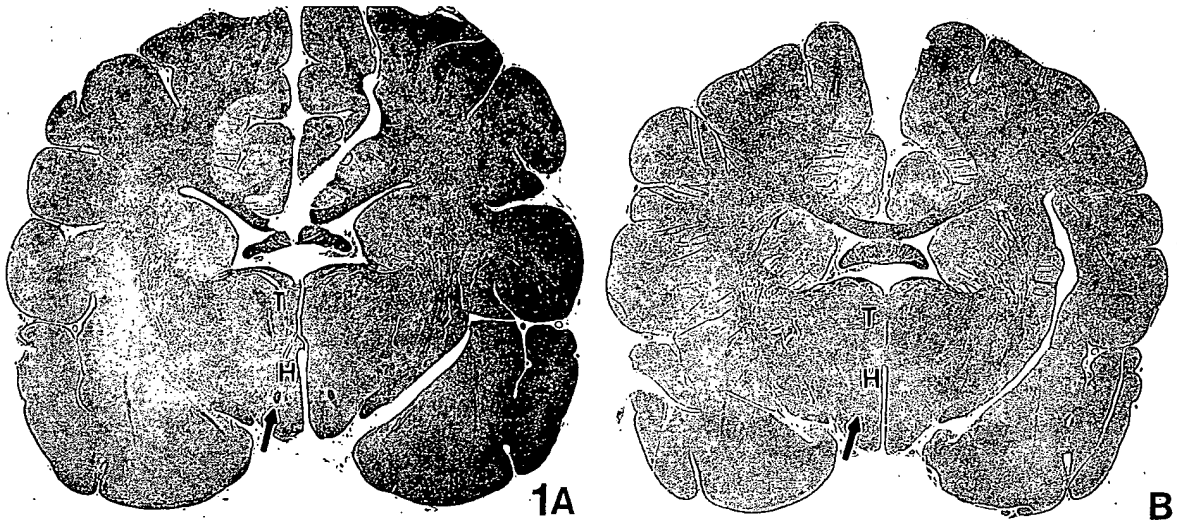

FIG. 1.—Coronal brain sections of A) a control dog and B) a dog treated with vigabatrin for 4 wk. Columns of the fornix (arrow), H (hypothalamus), and T (thalamus) are illustrated. Note, in the treated dog, the lack of staining involving the columns of the fornix that is consistent with the onset of vacuolation. H&E. ×2.5.

Clinical Pathology

Erythrocytic parameters were slightly affected in 5 treated dogs between 5 wk of treatment and 2 wk of recovery as indicated by slightly lower total erythrocyte counts ($<5.0 \times 10^6/mm^3$), hemoglobin (<14.0 g/dl), and hematocrit (<40%). Leukocyte counts, coagulation times (prothrombin; partial thromboplastin), and platelet counts were within normal limits.

Serum ALT values were generally decreased for the treated dogs for the 12 wk of vigabatrin administration. This effect is most likely due to nonspecific inhibition of other transaminase enzymes and is consistent with previous observations of transitory declines in the ALT enzyme in dogs and monkeys treated with ≥50 mg/kg/day of the drug for at least 3 mo (9). Slightly elevated cholesterol levels (216–279 mg/ml) were also detected in 50% of the dogs during the first 3 wk of treatment, possibly indicating fat mobilization in response to drug-induced anorexia. Increased cholesterol values were infrequent thereafter. All other clinical chemistry parameters appeared unaffected by treatment.

Histopathology

Like previous dog studies (4, 9, 10), varying degrees of vacuolation (intramyelinic edema) were observed in selected tracts of the brain (Table I) without any obvious sex differences. Many of the affected areas were present in section through the optic chiasma (Fig. 1). The progression of the vacuolation is best illustrated by the columns of the fornix, a tract very susceptible to vigabatrin effects (Fig. 2). When the columns of the fornix were analyzed, the amount of drug-related vacuolation was first statistically different ($p \leq 0.05$) from controls after 4 wk of treatment (Fig. 3). Mean values for drug-induced vacuolation of the hypothalamus first exceeded background threshold (0–2.0 severity grade) after 4 wk while the thalamus did not exceed background until after 5 wk. These findings led to the general conclusion that drug-induced vacuolation in more sensitive tracts could not be delineated from normal background until after 4 wk of treatment had been completed. In comparison, no difference was noted until after 5 wk, when all evaluated sites were analyzed collectively (Fig. 3). The severity of vacuolation continued to increase during the treatment period with maximum levels being found in the thalamus (3.3 mean severity grade) and columns of the fornix (5.0 mean severity grade) at week 11 and hypothalamus (3.3 mean severity grade) at week 8. In less sensitive myelinated tracts such as the optic tract, there was little difference between vacuolation in control and treated dogs (Table I). The spinal cord, peripheral nervous system (sciatic nerve and dorsal root, stellate, caudal cervical, and celiac ganglia), and eyes were unaffected by treatment.

The average severity grade of vacuolation among the various affected sites progressively declined beginning 1 wk postdosing (Figs. 3 and 4). No vacuolation was present in less sensitive areas such as the cortex, medial geniculated body, and habenular nucleus after 16 wk of recovery. In more sensitive areas (columns of the fornix, hypothalamus) the amount of vacuolation had returned to levels comparable to background in controls by the end of the

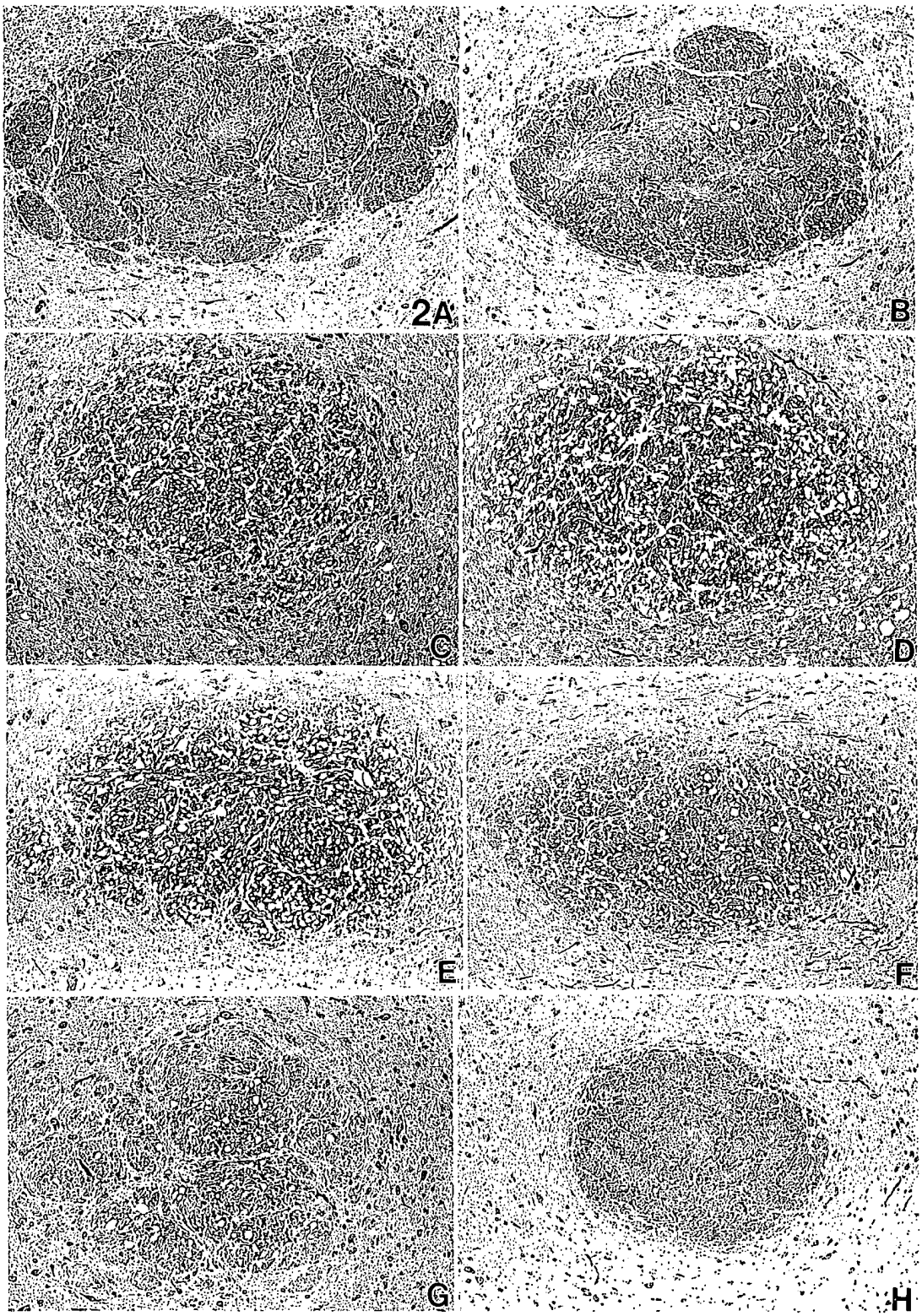

FIG. 2.—Extent of vacuolation in and around the columns of the fornix of dogs treated with 300 mg/kg/day of vigabatrin or allowed to recover from dosing. A) Control; B) 4 wk; C) 8 wk; D) 12 wk; E) 1 wk postdosing; F) 4 wk postdosing; G) 8 wk postdosing; H) 16 wk postdosing. H&E. ×40.

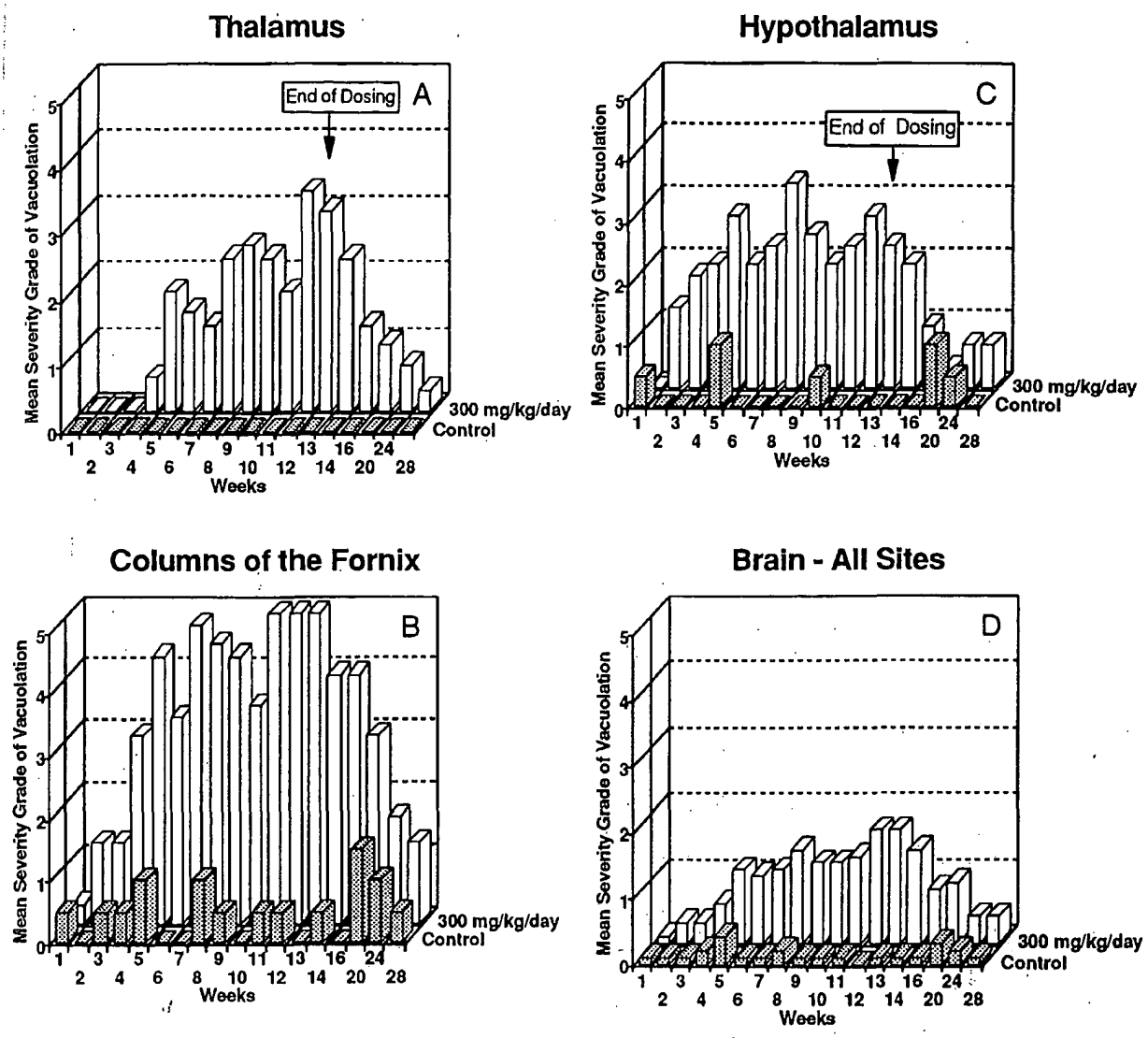

FIG. 3.—Comparison of severity grades of vacuolation in selected areas of the brain: A) thalamus; B) columns of the fornix; C) hypothalamus; D) average of all sites evaluated.

recovery period. These findings indicate that vigabatrin-induced vacuolation was reversible in the dog and are consistent with previous observations (9).

Concentrations of Plasma Vigabatrin and CSF Vigabatrin and GABA (24 hr Postdosing)

Steady-state plasma vigabatrin concentrations ranged between 4 and 17 nmol/ml during the 12 wk of treatment and were generally slightly higher in males (Fig. 4). After 1 wk of recovery (week 13), concentrations were below the limit of quantitation (1.6 nmol/ml). CSF vigabatrin concentrations reached steady-state levels after 1 wk of treatment and varied between 8 to 117 times higher (42–1,570 nmol/ml) than in plasma, reflecting slower clearance (Fig. 4). CSF total (free and conjugated) GABA concentrations for individual treated dogs were 4–32 nmol/ml during treatment compared to 0.1–0.6 nmol/ml in controls (Fig. 4). Concentrations returned to control levels following 1 wk of recovery from dosing.

There was no sampling of the 2 dogs that died. However, plasma and CSF samples were obtained from 1 male dog that was sacrificed moribund on day 24, approximately 27 hr after dosing. Plasma concentration of vigabatrin in this dog was 93 nmol/ml, which was the highest value detected in any of the treated dogs. CSF concentrations of vigabatrin and GABA were also markedly elevated, being 628 and 24.4 nmol/ml, respectively. The additional observation that mean plasma and CSF vigabatrin as

well as CSF GABA were elevated to their highest levels during the first few weeks of treatment suggests that the drug did cause the anorexia, which led to the generalized toxicity. The fact that force feeding allowed the dogs to overcome these effects and for the blood levels to decline somewhat indicates that the dogs were able to adapt to the high dose of 300 mg/kg/day of vigabatrin.

Analysis of Brain GABA, GABA-T, and GAD

After 1 wk of dosing, brain GABA levels were markedly elevated while GABA-T and GAD values were noticeably lower in all treated dogs (Fig. 5). GABA-T activity did not decrease noticeably after the first 2 wk of treatment. This may explain why brain GABA levels, which reached highest levels after 2 wk of treatment, did not increase further. Following cessation of dosing, brain GABA-T and GAD activities and GABA levels in vigabatrin-treated dogs became indistinguishable from controls after 1 wk and remained so during the remainder of the recovery period (Fig. 5).

DISCUSSION

Doses of vigabatrin, generally ranging between 35 and 50 mg/kg/day, have proven to be very efficacious in the treatment of human patients with a history of uncontrolled or drug-resistant epilepsy (3, 12, 29). Currently, there is no evidence of alterations in clinical (3, 11, 12, 22, 29, 35, 36), electrophysiological (14), and psychometric and psychophysiological tests (30), and brain histology (6, 10, 12, 15, 27) in these patients. However, findings in preclinical animal studies have caused safety concerns about long-term clinical use of vigabatrin. These studies demonstrated that microscopic brain vacuolation (intramyelinic edema) developed in a dose-related (50–300 mg/kg/day) manner in treated rats, mice, and dogs but was equivocal in monkeys given 300 mg/kg/day (1, 5, 9, 10). Similar vacuolation has been detected in animals treated with other GABA-T inhibitors including ethanolamine-*o*-sulfate (16), γ-allenyl GABA (9), and BW 357U (9). The issue of safety has led to an ongoing effort to validate noninvasive procedures to detect this vacuolation. However, the precise time when vigabatrin-induced brain vacuolation develops has not been established. The principal objective of the present investigation was to establish the time-course for the onset and reversibility of brain vacuolation using the dog. It is a species very susceptible to vigabatrin's effects and is a relatively good model to study evoked potentials (1, 32) and MRIs (37). A high dose of 300 mg/kg/day was chosen to insure that detectable changes in both brain histology and biochemistry would be present after 12 wk of treatment (9, 32). To avoid bias, the histological evaluation of dog brains was conducted in a blinded fashion so that the dose and week of treatment or recovery were unknown to the reviewing pathologists. A second objective was to attempt to correlate

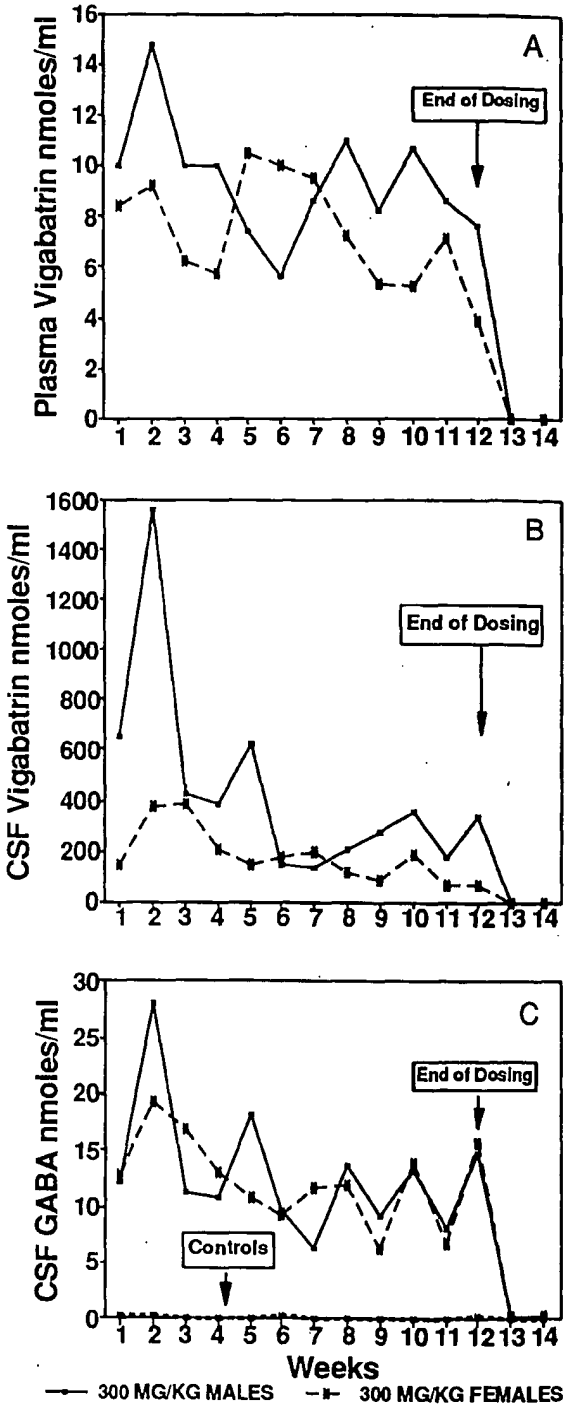

FIG. 4.—Pharmacokinetic analysis of A) plasma vigabatrin, B) CSF vigabatrin, and C) CSF GABA.

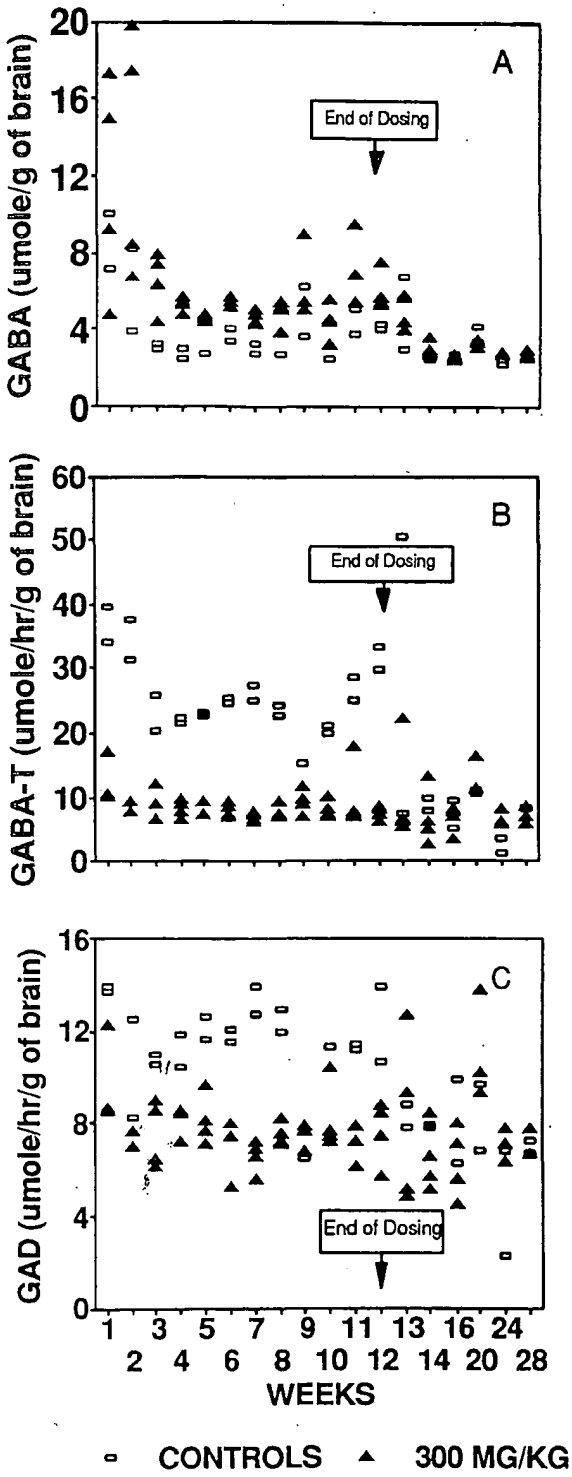

FIG. 5.—Effects of vigabatrin on levels of brain A) GABA, B) GABA-T, and C) GAD.

the vacuolation (intramyelinic edema) to other drug-induced clinical and biochemical effects.

The first indication of drug effects was clinical signs of anorexia and emesis seen during the first week of treatment. At the end of 1 wk of dosing, plasma and CSF vigabatrin as well as CSF GABA concentrations 24 hr postdosing were at study-state levels. Brain biochemistry changes were also apparent after the first week. Lowered GAD activity was most likely due to feedback inhibition of new enzyme synthesis because vigabatrin does not inhibit this enzyme *in vitro* (18–20). Since reduced GAD activity should decrease GABA synthesis, the elevated GABA levels were indicative of vigabatrin's effect on prolonging GABA degradation by its profound inhibition of GABA-T. Previous studies have indicated that GABA-T inhibitors must inhibit enzyme levels by about 50% in order to significantly elevate GABA in rodents (8, 17). Such was the effect in the dogs after dosing vigabatrin for 1 wk. After reaching highest levels after 2 wk of treatment, GABA and GABA-T values were detected at slightly lower but relatively constant levels for the remainder of treatment. By the fourth week of treatment, microscopic differences between background vacuolation and drug-related vacuolation were apparent and continued to increase until plateauing in severity between 8 and 12 wk of dosing. The magnitude and progression of vacuolation appeared to be directly related to the sensitivity of the affected tract or area of the brain. At approximately the same time interval of 4 wk, clinical signs began to dissipate in frequency and severity, probably the result of adaptation to the drug as suggested by trends in both brain biochemistry and pharmacokinetics. A recent investigation of dogs treated with the 300-mg/kg/day dose of vigabatrin suggested that slowing of somatosensory and visual-evoked potentials began at week 6 and weeks 4–6, respectively. However, significant differences based on group averages were not observed until 8–10 wk of dosing (32). MRI changes were also detectable after 4–7 wk (37). Thus, functional disturbances of the vigabatrin-treated dog brain appear to occur at about the same time or slightly after the intramyelinic edema becomes distinguishable from the background vacuolation of controls. All of these drug-induced effects are reversible upon cessation of dosing, although brain histology was last to return to normal.

Although the cause of vigabatrin-induced microvacuolation is unknown, similar neuropathology has been reported with other GABA-T inhibitors such as ethanolamine-*o*-sulfate (16), γ-allenyl GABA (9), and BW 357U (9), suggesting that elevated GABA levels may be involved in the pathogenesis. In the present study, changes in brain biochemistry did not correlate simultaneously with the appearance or regression of the intramyelinic edema. These results suggest that elevations in brain GABA may be responsible for the formation of intramyelinic edema

but that additional mechanisms may be involved in its development. The absence of vacuolation in the retina, optic nerve, spinal cord, and peripheral nervous system including sciatic nerve and both somatic and autonomic ganglia is consistent with findings of previous animal studies that vigabatrin's neuropathology is confined to the brain (4, 9, 10).

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Pharmacokinetics of oxcarbazepine in the dog

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Schicht, S., Wigger, D. & Frey, H.-H. Pharmacokinetics of oxcarbazepine in the dog. *J. vet. Pharmacol. Therap.* 19, 27–31.

Oxcarbazepine has been proven to be a promising new antiepileptic drug for the treatment of human epilepsy. Unlike carbamazepine, it is not oxidatively metabolized in humans, and therefore causes almost no induction of hepatic enzymes at clinically effective dosages. Though showing similar efficacy to carbamazepine, it has been reported to cause significantly fewer side-effects. It was the purpose of the present study to determine whether oxcarbazepine might be suitable for the treatment of canine epilepsy. In single-dose experiments, 40 mg/kg oxcarbazepine as a suspension was administered to seven dogs via gastric tube. Plasma concentrations reached peak concentrations of 2.4–8.8 μg/mL at about 1.5 h and declined with an elimination half-life of approximately 4 h. The corresponding concentrations of its metabolite, 10,11-dihydro-10-hydroxycarbamazepine, did not exceed 1 μg/mL. During continued treatment for 8 days, doses of 30 and 50 mg/kg were administered orally in capsules to two dogs three times a day. Plasma concentrations showed a pronounced decline from day 3, and the terminal half-life decreased to 2 h and 1 h. This is considered to be the result of oxcarbazepine inducing its own metabolism. The data reveal that oxcarbazepine, compared with former results with carbamazepine, offers no advantage for the treatment of epileptic dogs.

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INTRODUCTION

Oxcarbazepine has proved to be a promising new antiepileptic drug for the treatment of human epilepsy. It has been recommended for patients suffering from partial and generalized tonic–clonic seizures (Jensen & Dam, 1990). Unlike carbamazepine, it is not oxidatively metabolized in humans and therefore causes almost no induction of hepatic enzymes at clinically effective dosages (Schwabe, 1994). Though showing similar efficacy, it has been reported to display significantly fewer side-effects (Dam *et al.*, 1989). In dogs the pharmacokinetics of carbamazepine was studied previously by Frey & Löscher (1980). Following single oral dose administration, plasma concentrations of carbamazepine declined rapidly with an elimination half-life of 1.5 h. Elimination was even accelerated following multiple dosing over several days, because plasma concentrations showed a pronounced and progressive decrease from day 2 and half-lives decreased to 0.7 h. The explanation was a rapid induction of metabolizing enzymes in the liver. Thus, carbamazepine was not considered to be suitable for the therapy of epileptic disorders in dogs.

It was the purpose of the present study to determine whether oxcarbazepine might be useful in the treatment of canine epilepsy.

MATERIALS AND METHODS

Subjects

Ten dogs of both sexes, one German shepherd, four mongrels (boxer/Foxhound) and five Beagles, aged 5–12 years and weighing between 14 and 32 kg, were used in the experiments.

Single-dose experiments

The pharmacokinetic data of oxcarbazepine and its metabolite GP 47779 (10,11- dihydro-10-hydroxycarbamazepine) were determined after administration of 40 mg/kg oxcarbazepine, suspended in 10 mL 0.5% methylcellulose via gastric tube. Blood samples for analysis were taken before as well as at 15, 30, 45, 60 and 90 min and 2, 3, 4, 5, 6, 8, 12 and 24 h after drug administration. For the calculation of the pharmacokinetic parameters, the methods and symbols according to Baggot (1977) were used and verified by TopFit, a pharmacokinetic and pharmacodynamic data analysis system for the PC, version 2.0.

Multiple-dose experiments

In two dogs the pharmacokinetic data were determined after oral administration of oxcarbazepine (30 and 50 mg/kg) in capsules administered three times a day (07.00, 15.00 and 23.00 hours) for 1 week. Blood samples for analysis were taken three times a day (07.00, 09.00 and 11.00 hours) on seven consecutive days. On the eighth day oxcarbazepine, suspended in 10 mL of 0.5% methylcellulose, was given only once via gastric tube at 07.00 hours and blood samples were taken as in the single-dose experiments to determine the terminal elimination half-life of the drug.

Passage of oxcarbazepine into cerebrospinal fluid (CSF)

Three dogs were anaesthetized by intravenous injection of 30 mg/kg pentobarbital and were relaxed by infusion of succinylcholine at a dose of 0.01 mg/kg/min following an initial bolus of 0.1 mg/kg. The dogs were intubated (Rüsch tracheotubes of adequate size) and ventilated by an Assistor 641 (Drägerwerk, Lübeck, Germany). Arterial blood pressure and heart rate were recorded on a multichannel recorder. Anaesthesia was maintained by small additional doses of pentobarbital when the blood pressure started to rise, which was chosen as a sign of nociception returning during immobilization by succinylcholine. Blood samples were taken from a jugular vein and cerebrospinal fluid (CSF) samples from a cannula introduced into the cisterna magna. Details of the preparation have been described previously (Frey & Löscher, 1978; Frey *et al.*, 1979). A dose of 40 mg/kg oxcarbazepine was administered through a polyethylene catheter into the jejunum. Blood and CSF samples were taken before as well as 15, 30, 45, 60 and 90 min and 2, 2.5, 3, 3.5 and if possible 4 and 4.5 h after drug administration. The permeability constants were calculated using the equation given by Mayer *et al.* (1959):

$$P\,(\text{min}^{-1}) = -1/t \ln \frac{C_s - C_f}{C_s}$$

where C_s = is the concentration of free drug in serum and C_f = concentration of free drug in liquor.

After 4.5 h at the latest, the experiment was terminated with an overdose of pentobarbital.

Binding of oxcarbazepine and GP 47779 to serum proteins

The binding of both drugs to serum proteins was determined by equilibrium dialysis using a Dianorm apparatus (Diachema, Rüschlikon, Switzerland) with 2 mL of cells and cellulose membranes (Visking, Union Carbide, Chicago, IL, USA). Dialysis was performed for 16 h at 37 °C against 0.067 M phosphate buffer adjusted to pH 7.4. Fresh serum was collected from six dogs and divided into six samples each. Oxcarbazepine and GP 47779, at three different concentrations of 5, 10 and 20 μg/mL and 0.1, 0.5 and 1 μg/mL, respectively, were added and the analysis was performed. The percentage bound was calculated from the drug concentrations determined in serum and buffer phase according to the following equation:

$$\text{Percent bound} = \frac{\text{concentration in serum} - \text{concentration in buffer}}{\text{total concentration in serum}} \times 100$$

Determination of oxcarbazepine and GP 47779 in plasma and CSF

Determination was carried out by means of a self-developed high-performance liquid chromatographic (HPLC) method. The HPLC apparatus (Knauer) consisted of an injection valve (Reodyne, model 7125, CA, USA), a pump (Knauer, model 64, Bad Homburg, Germany), an integrator (Shimadzu, model C-R3A, Chromatopac, Kyoto, Japan), a spectral photometer model no. 731.87 00 000 (Knauer), a pre-column (40 × 4.6 mm) and a column (250 × 4.6 mm), both filled with spherisorb ODS II, 5 m (Optilab, Berlin, Germany). The eluent consisted of 62% phosphate buffer, 35% acetonitrile and 3% tetrahydrofuran. The phosphate buffer consisted of 30 mM KH_2PO_4 adjusted to pH 4 with phosphoric acid. For the preparation of the eluent, bidistilled water was used. Carbamazepine, dissolved in eluent, acted as internal standard and was added to the plasma samples to give a concentration of 5 μg/mL. Thereafter 1.5 mL of chloroform was added and the samples were shaken for 1 min. Then the phases were seperated by centrifugation. Afterwards 1 ml of the chloroform phase was removed by piercing through the solid plasma protein layer on top of it and placed in another vessel. Chloroform was evaporated on a water bath of 40°C under constant nitrogen flow. The residue was reconstituted in 100 μL of the eluent. The column was injected with 20 μL of this solution. The flow rate was 1 mL/min and photometric measurement was performed at a wavelength of 210 nm. The level of quantification of the assay method was 0.03 μg/mL.

Drugs used

Oxcarbazepine as a powder was suspended in 10 mL of 0.5% methylcellulose. For the multiple-dose experiments oxcarbazepine was filled into acid-soluble gelatin capsules.

RESULTS

Single-dose experiments

Following intragastric administration of the oxcarbazepine suspension, maximal plasma concentrations of 2.37–8.79 μg/mL (median $\tilde{x}$ = 6.32 μg/mL) were reached at 1.9 h. The elimination half-life ($t_{1/2\beta}$) varied between 1.6 and 7.8 h ($\tilde{x}$ = 4.0 h). In three dogs the plasma concentration curve fitted a two-compartment model and distribution half-lives ($t_{1/2\alpha}$) of 1.4, 2.5 and 2.8 h were obtained. After 2.6 h maximal concentrations of the metabolite ranged between 0.30 and 1.04 μg/mL ($\tilde{x}$ = 0.38 μg/mL) (see Table 1 and Figs 1 and 2).

Multiple-dose experiments

During continued treatment with doses of 30 and 50 mg/kg three times a day, respectively, a decline in the plasma concentrations

Table 1. Single-dose experiments: pharmacokinetic data of oxcarbazepine and its metabolite GP 47779 after administration of 40 mg/kg oxcarbazepine via a gastric tube to seven dogs.

	Oxcarbazepine							GP 47779 (10,11-dihydroxy-10-hydroxycarbamazepine)				
Dog no.	C_{max} (μg/mL)	t_{max} (h)	k_a (h^{-1})	k_d (h^{-1})	k_e (h^{-1})	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	C_{max} (μg/mL)	t_{max} (h)	k_f (h^{-1})	k_e (h^{-1})	$t_{1/2\beta}$ (h)
1	8.79	3.00	1.04	–	0.17	–	4.01	1.04	6	0.45	0.18	3.95
2	4.31	1.5	1.84	0.25	0.09	2.83	7.74	0.32	3	1.05	0.06	12.08
3	6.32	3	1.27	–	0.18	–	3.87	0.38	2	1.11	0.12	5.66
4	7.70	2	2.65	–	0.23	–	3.03	0.47	3	1.03	0.20	3.45
5	8.30	1.5	1.51	0.28	0.16	2.46	4.28	0.61	2	1.55	0.27	2.54
6	2.37	1.5	2.31	0.51	0.09	1.37	7.76	0.34	1.5	–	–	–
7	3.99	1	0.77	–	0.44	–	1.57	0.30	1	1.36	0.23	3.01
$\tilde{x}$	6.32	1.5	1.51		0.17		4.01	0.38	2	1.08	0.19	3.7

The drug was given as a suspension in 0.5% methylcellulose. The table presents the median ($\tilde{x}$). C_{max} = maximal plasma concentration, t_{max}, time of peak concentration; k_a, absorption constant; k_d, constant of distribution; k_e, constant of elimination; K_f, constant of formation of the metabolite; $t_{1/2\alpha}$ = distribution half-life; $t_{1/2\beta}$, elimination half-life.

occurred on day 3 in both dogs. The terminal half-lives decreased from 5 to 2 h and from 7 to 1 h (see Fig. 3).

Binding of oxcarbazepine and its metabolite to serum proteins

In the range of concentrations observed during clinical trials, about 45% of oxcarbazepine and 53% of GP 47779 were bound to the proteins of dog serum (Table 2).

Passage of oxcarbazepine into CSF

In acute experiments with three anaesthetized dogs, permeability constants (P) of 0.042, 0.014 and 0.011/min were calculated for the passage of oxcarbazepine into the CSF. A steady-state drug concentration in CSF was reached within 1 h after intrajejunal administration (see Fig. 4).

Side-effects

Side effects of oxcarbazepine (e.g. sedation, nausea, anorexia, weight loss, ataxia), especially during the multiple dose experiments, were not observed with the doses employed in this study.

DISCUSSION

After single-dose oral administration oxcarbazepine was absorbed by dogs at a rate of about 1.6 h^{-1}, which is similar to that found for carbamazepine by Frey & Löscher (1980). A liquid formulation of carbamazepine was absorbed at a rate of 1.8 h^{-1} and carbamazepine tablets at 1.3 h^{-1}. Maximal plasma concentrations of oxcarbazepine were reached at about 1.9 h. For carbamazepine maximum plasma concentrations were obtained at 1.0 h and 2.1 h for the liquid and the tablets respectively (Frey & Löscher, 1980).

In the single-dose experiments a dose of 40 mg/kg oxcarbazepine caused varying maximal plasma concentrations ranging from 2.37 to 8.79 μg/mL. For carbamazepine plasma concentrations between 6 and 12 μg/mL are kown to be 'therapeutic' for man. Provided these values are valid also for dogs, and because oxcarbazepine, administered at a 50% higher dosage than carbamazepine, is believed to possess an anticonvulsant potency equal

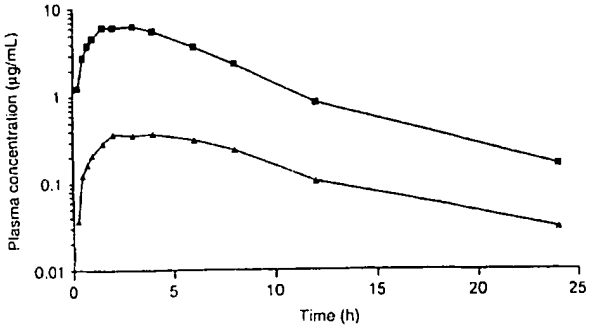

Fig. 1. Plasma concentration curves of oxcarbazepine (■) and its metabolite (▲) in a dog fitting a one-compartment model. The drug, at 40 mg/kg, suspended in 0.5% methylcellulose, was administered via a gastric tube.

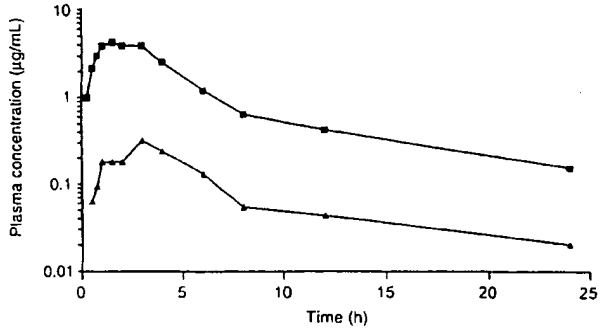

Fig. 2. Plasma concentration curves of oxcarbazepine (■) and its metabolite (▲) in a dog fitting a two-compartment model. 40 mg/kg of the drug, suspended in 0.5% methylcellulose, were administered via a gastric tube.

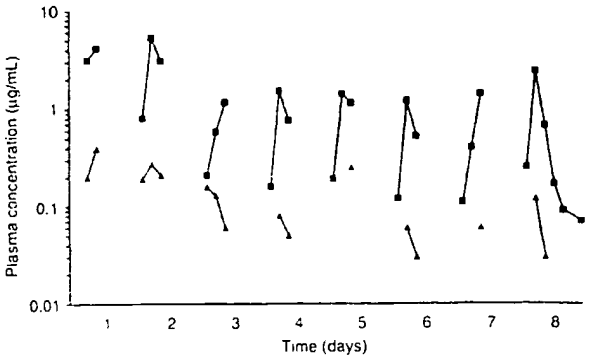

Fig. 3. Plasma concentrations of oxcarbazepine (■) and its metabolite (▲) in a dog which was treated for 1 week with three daily doses of 30 mg/kg oxcarbazepine (capsules). On the eighth day, oxcarbazepine was given as a suspension in the morning via gastric tube to determine the terminal half-life. For each of the seven previous days, plasma concentrations determined before and 2 and 4 h after the 07.00 hours dose are depicted.

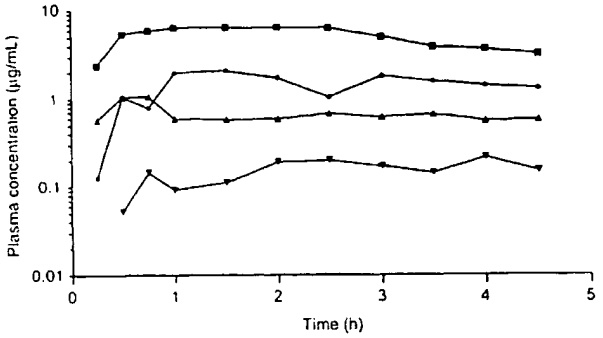

Fig. 4. Plasma and CSF concentrations of oxcarbazepine (plasma, ■; CSF, ●) and its metabolite (plasma, ▲; CSF, ▼) after intrajejunal administration of 40 mg/kg to a dog held under general anaesthesia over 4.5 h.

to that of carbamazepine (Baltzer & Schmutz, 1978; Dam *et al.*, 1989), the plasma concentrations reached in this study might lie at the lower limit of the therapeutic range for the dog.

In the dogs used in our experiments the elimination half-life of oxcarbazepine was 4.6 h on average, which is only one-half to one-third the value determined for man (Jensen & Dam, 1990), indicating a higher rate of metabolism in dogs. Nevertheless, the elimination of oxcarbazepine is slower than that of carbamazepine, for which an elimination half-life of about 1.5 h has been reported (Frey & Löscher, 1980).

In three dogs the plasma concentration curve fitted a two-compartment model and distribution half-lives of 1.4, 2.5 and 2.8 h were obtained. The elimination half-lives in these dogs tended to be longer, reaching 4.3, 7.7 and 7.8 h. No further attempts were made to identify the deep compartment in these individuals, from which oxcarbazepine was eliminated more slowly.

The main metabolite of oxcarbazepine in humans, 10,11-dihydro-10-hydroxycarbamazepine (GP 47779), which possesses anticonvulsant activity similar to the parent compound (Baltzer & Schmutz, 1978), occurred in dogs at concentrations of about 0.38 µg/mL on average and did not exceed 1.04 µg/mL. In an earlier study Feldmann *et al.* (1978) could not even detect this metabolite in dogs after oral dosing of 5 mg/kg. The concentration measured in our experiments is rather low compared with that found in humans, in whom GP 47779 rapidly reached concentrations in the range 2–10 µg/mL. Immediately following administration to humans, oxcarbazepine was extensively converted to the metabolite to such a degree that no more than 2 µg/mL of the parent compound could be detected (Jensen & Dam, 1990). This discrepancy might be explained to some extent by the fact that in dogs GP 47779 is partly (re-)metabolized to oxcarbazepine, whereas this process is negligible in humans (Feldmann *et al.*, 1978).

Table 2. Binding of oxcarbazepine and GP 47779 to serum proteins of dogs

	Concentration in serum (µg/mL)	Proportion bound (%) $\bar{x}$ (± SD)
Oxcarbazepine	5	47.0 (± 4.8)
	10	47.5 (± 2.7)
	20	40.5 (± 4.4)
GP 47779	0.1	53.1 (± 1.8)
	0.5	54.3 (± 3.3)
	1	53.6 (± 5.6)

Determination was performed by equilibrium dialysis at 37°C using fresh serum samples from six dogs.

In our experiments the elimination half-life for GP 47779 was about 3.7 h after oral administration of oxcarbazepine. This value differs considerably from the 1.4 h found by Feldmann *et al.* (1981) after intravenous injection of GP 47779 alone. However, in the latter study elimination of GP 47779 started immediately, whereas in our experiments it had to be formed by biotransformation of oxcarbazepine.

During continued treatment of two dogs with oxcarbazepine for 8 days, a rapid and pronounced decline in the plasma concentrations of the drug itself and its metabolite to about 50% of the original plasma levels occurred as early as the third day of treatment, and terminal half-lives decreased from 5 to 2 h and from 7 to 1 h. The explanation is probably an induction of metabolizing enzymes in the liver microsomes, as is well known for carbamazepine in rats (Wagner & Schmid, 1987), dogs (Faigle *et al.*, 1976, Frey & Löscher, 1980), monkeys (Patel *et al.*, 1978; Lockard *et al.*, 1979) and man (Eichelbaum *et al.*, 1975; Morselli *et al.*, 1975; Faigle *et al.*, 1976). Induction of hepatic enzymes by oxcarbazepine has already been reported in rats (Wagner & Schmid, 1987), but not following repeated dosing in humans with therapeutic dosages (Feldmann *et al.*, 1981; Schwabe, 1994).

The permeability constants for the passage of oxcarbazepine into the CSF correspond to those previously found by Frey & Löscher (1980) for carbamazepine and carbamazepine 10,11-epoxide. The CSF concentrations of oxcarbazepine and GP 47779 at steady state corresponded to the concentrations of the free drugs in plasma.

This is in accordance with serum protein binding of oxcarbazepine and its metabolite which was about 50%. Jensen & Dam (1990) reported serum protein bindings of 66% for oxcarbazepine and 27–28% for GP 47779 in dogs without giving methodological or bibliographic references.

The data reveal that, in contrast to humans, oxcarbazepine does induce its own metabolism in the dog and is eliminated fairly rapidly. Compared with results obtained for carbamazepine (Frey & Löscher, 1980), the drug offers no advantage for the treatment of epileptic dogs.

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